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Vitamins and Aging: Pathways to NAD⁺ Synthesis

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Recent genetic evidence reveals additional salvage pathways for NAD⁺ synthesis. In this issue, Belenky et al. (2007) report that nicotinamide riboside, a new NAD⁺ precursor, regulates Sir2 deacetylase activity and life span in yeast. The ability of nicotinamide riboside to enhance life span does not depend on calorie restriction.

The water-soluble vitamin niacin (B3) is a building block for NAD⁺ (nicotinamide adenine dinucleotide), which is a carrier of two electron equivalents for the oxidation of carbohydrates during ATP synthesis. Although this redox role of NAD⁺ is well established, there is renewed interest in NAD⁺ metabolism due to recent evidence showing that NAD⁺ regulates diverse pathways including those controlling life span (Ziegler and Niere, 2004). The broad-ranging importance of niacin metabolites to cellular function is further underscored by recent work providing genetic evidence for the existence of several additional pathways for salvaging the nicotinamide ring of NAD⁺ (Belenky et al., 2007; Bieganowski and Brenner, 2004).

Belenky et al. (2007) now demonstrate that a newly described NAD⁺ precursor, nicotinamide riboside (NR) (Bieganowski and Brenner, 2004), contributes to cellular NAD⁺ levels through at least two pathways in the budding yeast *Saccharomyces cerevisiae* (Figure 1). Both pathways salvage the nicotinamide ring for entry into established path-

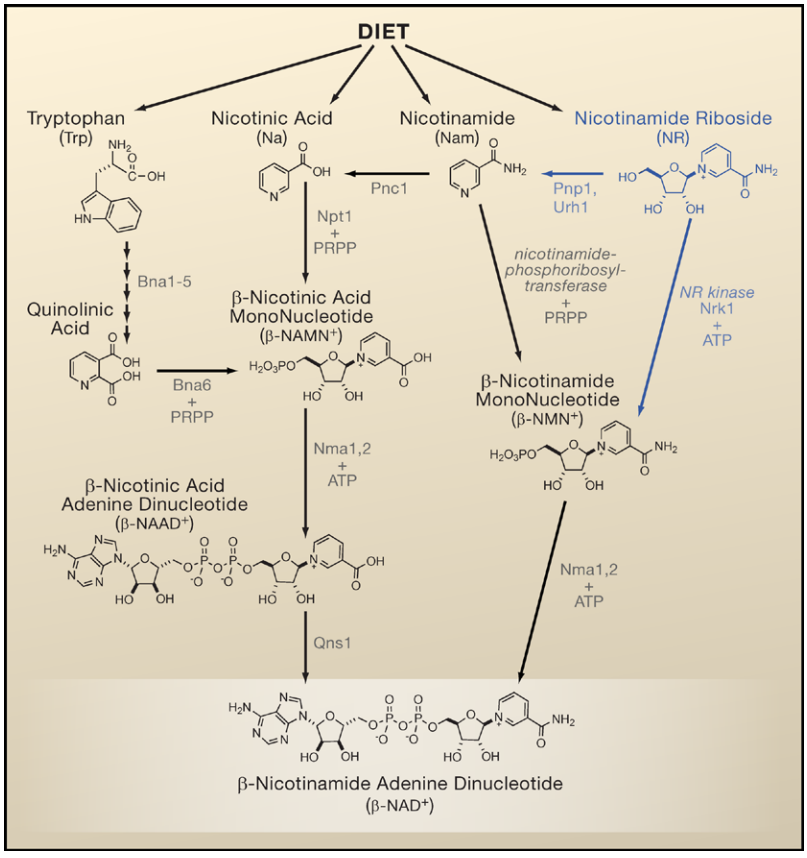

Figure 1. Pathways to NAD⁺ Synthesis
NAD⁺ can be generated by the de novo pathway starting with the precursor tryptophan or by the salvage pathway from the precursors nicotinamide (Nam), nicotinic acid (Na), and nicotinamide riboside (NR). All of these precursors are available from the diet. Niacin often refers to two related compounds, nicotinamide (niacinamide) and nicotinic acid. The yeast gene encoding the enzyme that catalyzes each step of NAD⁺ biosynthesis is indicated; recently discovered portions of the salvage pathway are shown in blue. One arm of the salvage pathway requires the phosphorylation of NR to nicotinamide mononucleotide (NMN) by the NR kinase Nrk1. The other pathway involves the cleavage of the NR glycosidic bond by Pnp1 (a phosphorylase) and Urh1 (a hydrolase) to yield free nicotinamide. Whereas higher eukaryotes can readily convert nicotinamide to NMN through the action of the salvage pathway enzyme nicotinamide phosphoribosyl transferase (Revollo et al., 2007), yeast primarily hydrolyze nicotinamide to nicotinic acid through the nicotinamidase Pnc1 (Anderson et al., 2003). Curiously, mammals do not appear to have a nicotinamidase. (Bna6, quinolinate phosphoribosyl transferase; Qns1, NAD synthetase; Nma, nicotinate mononucleotide adenyltransferase; Npt, nicotinate phosphoribosyl-transferase; PRPP, phosphoribosyl pyrophosphate.)

ways of NAD^+ synthesis. One pathway requires the phosphorylation of NR to nicotinamide mononucleotide (NMN) by the NR kinase Nrk1 (Figure 1). NMN is then readily converted to NAD^+ by NMN adenylyltransferase, a well-known enzyme of the NAD^+ salvage pathway. The second pathway involves the cleavage of the NR glycosidic bond to yield free nicotinamide (Figure 1). Belenky et al. demonstrate that NR can replace nicotinic acid as a vitamin to stably maintain intracellular levels of NAD^+ levels during growth of yeast in liquid cultures (Belenky et al., 2007). Utilization of NR requires either an active Nrk1 or an active Urh1/Pnp1 pathway. In the absence of NR supplementation, the Nrk1 and Urh1/Pnp1 pathways contribute to NAD^+ levels, suggesting that endogenous NR may be produced in the NAD^+ salvage pathway under normal growth conditions.

The authors link the ability of NR to induce NAD^+ synthesis with increased activity of Sir2 (silent information regulator 2) (Belenky et al., 2007). Sir2 is an NAD^+-dependent histone deacetylase involved in gene silencing at telomeres, reducing the recombination of ribosomal DNA (rDNA) and increasing life span (reviewed in Haigis and Guarente, 2006). Yeast strains that exhibit lower levels of NAD^+ display aberrant gene silencing and age rapidly. Although caloric restriction is a well-established means of increasing life span in diverse organisms, the Belenky et al. study indicates that NR can extend yeast life span through activation of pathways that respond to increased NAD^+, such as those dependent on Sir2.

In mammals, Sir2-related enzymes (the sirtuins) are implicated in cell survival and the metabolism of fatty acids and glucose (Haigis and Guarente, 2006). Several intriguing studies suggest that enhanced NAD^+ synthesis is associated with increased sirtuin function (references cited in Belenky et al., 2007). However, what remains to be determined is how sirtuins actually respond to increased NAD^+ synthesis. It is unclear how an increase in intracellular NAD^+ from 1 to 2 mM leads to substantial activation of Sir2 (Belenky et al., 2007), whose K_m values are reported in the μM range (reviewed in Grubisha et al., 2005). Belenky et al. propose that the 2-fold increase in NAD^+ through vitamin supplementation is used for discretionary pathways, such as those mediated by Sir2. However, it is difficult to imagine how the cell distinguishes essential versus nonessential NAD^+ utilization, unless there were spatially distinct pools of NAD^+. Another possibility is that NAD^+-synthesizing enzymes might physically associate and directly channel NAD^+ into the active site of sirtuins. The existence of NAD^+ protein sensors that respond to appropriate levels of NAD^+ and modulate sirtuin activity is another unexplored possibility. Moreover, as has been postulated for nicotinamide (Anderson et al., 2003), increased niacin/NR salvage may deplete intermediates that inhibit or oppose the function of sirtuins, such that it is the derepression of sirtuins and not NAD^+ synthesis per se that is responsible for net activation.

It may not be surprising that cells harbor multiple strategies to salvage niacin, an essential molecule for all living organisms. Under conditions that result in activation of enzymes that increase cleavage of NAD^+, the cell may protect itself from a catastrophic loss of NAD^+ by increasing expression/activity of NAD^+ salvage enzymes. Consistent with this notion, the yeast nicotinamidase Pnc1 is one of the most highly expressed genes in response to various cellular insults (Anderson et al., 2003). Also, expression of NR kinase mRNA is highly upregulated 2 weeks after sciatic nerve transection in rats (Sasaki et al., 2006). Therefore, it is appealing to suggest that NAD^+ salvage enzymes are tightly linked through bidirectional regulatory circuits with NAD^+-consuming enzymes such as sirtuins and poly (ADP-ribose) polymerases. Nutritional status and the availability of niacin metabolites may coordinately modulate the levels and activity of these enzymes. Salvage pathways might be especially crucial in postmitotic cells (such as neurons) where NAD^+ salvage might predominate over de novo synthesis, which likewise might prevail in actively dividing cells. Further studies will need to address the importance of NR in human health and determine whether NR could be an alternative niacin supplement/vitamin to treat several age-related afflictions such as diabetes, atherosclerosis, neurodegeneration, and cancer.

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